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Abundant expression of zinc transporters in Bergman glia of mouse cerebellum

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Abstract

Zinc transporters (ZnTs) are membrane proteins involved in zinc ion transportation in mammalian cells. Seven members of ZnT family, ZnT1–7, have been cloned and characterized. These transporter proteins have different cellular and sub-cellular locations, suggesting that they may play different roles in zinc homeostasis in normal and pathological conditions in different tissues. Cerebellum is one of the most zinc-enriched regions in the central nervous system, but little is known about zinc metabolism in the cerebellum. In the present study, we investigated the detailed distributions of four members (ZnT1, ZnT3, ZnT4 and ZnT6) of the ZnT family, in the mouse cerebellum. Immunostaining and confocal microscopic observations revealed a similar staining pattern of ZnTs in the molecular layer and the Purkinje cell layer. Double labeling with anti-S-100β or anti-MAP2 and anti-ZnTs clearly showed that the Bergman glial cell bodies in the Purkinje cell layer and their radial processes in the molecular layer exhibited strong immunofluorescence of all the tested ZnTs. However, the somata of the Purkinje cells contained a moderate immunostaining for ZnT1, but virtually lack of other three ZnTs. In the granular layer, ZnTs appeared with different immunostaining patterns. ZnT1 was expressed in a small number of neuronal cell bodies and their primary dendrites, whereas ZnT3 and ZnT4 were present in nerve terminals but not in the neuronal somata. ZnT6 was undetectable in either the cell bodies or processes in the granular layer. The present results indicate that the Bergman glial cells may play an important role in zinc metabolism in the mouse cerebellar cortex.

Keywords: Astrocyte; Autometallography (AMG); Confocal laser scanning microscopy; Immunohistochemistry; S-100; Zinc transporter

1. Introduction

Zinc, after iron, is the second most abundant trace element in the mammalian tissue, and is essential for development, growth, DNA synthesis, immunity, and a wide array of cellular processes [4,5,56,62]. It is known that most zinc is tightly bound to macromolecules to maintain the three-dimensional structure of a wide variety of proteins or to serve as a cofactor of a large number of enzymes [6,8]. A small amount of zinc ions are free or weakly bound to the host molecules, and they are chelatable with zinc specific fluorescence dyes, such as zinquin [64] and 6-methoxy 8-para toluene sulfonamide quinoline (TSQ) [23], and histochem-

Abbreviations: AD, Alzheimer's disease; AMG, autometallography; AMPA, α-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BSA, bovine serum albumin; CLSM, confocal laser scanning microscopy; CNS, central nervous system; GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; IR, immunoreactivity; MAP, microtubule-associated protein; MT, metallothionein; NDS, normal donkey serum; NGS, normal goat serum; NMDA, *N*-methyl-D-aspartate; PBS, phosphate buffered saline; TBS, Tris–HCl buffered saline; TSQ, 6-methoxy 8-para toluene sulfonamide quinoline; ZEN, zinc-enriched neuron; ZnT, zinc transporter

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ically detectable with Timm staining or autometallography (AMG) [12–14].

In the central nervous system (CNS), the histochemically detectable or chelatable zinc (approximately, 10–15% of total brain zinc) is mainly located in the synaptic vesicles of a subset of neurons [22,24]. Neurons that contain free zinc ions in the vesicles of their pre-synaptic boutons have been termed zinc-enriched (ZEN) or zinc-containing neurons [15,21], or "zinc-ergic" neurons [3,37,61]. ZEN neurons have been found throughout the mammalian CNS, but appear to be most abundant in the telencephalon. Studies from the hippocampus, amygdala and neo-cortex revealed that ZEN neuron in the forebrain represent a subgroup of excitatory glutamatergic neurons [25,53]. However, recent studies have shown that inhibitory γ-aminobutyric acid (GABA)-ergic ZEN neurons exist in the spinal cord and the cerebellum [2,16,57,58,60]. Therefore, it has been hypothesized that vesicular zinc may contribute to synaptic neurotransmission and neuromodulation of the two major neurotransmitter systems, the glutamatergic and GABAergic systems. After release from pre-synaptic vesicles into the synaptic cleft zinc may modulate the postsynaptic receptors, including GABA, *N*-methyl-D-aspartate (NMDA) receptors, and α-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainite receptors [29,31,43,44,54].

Although, zinc is present in high amounts in the CNS, very little is known about zinc metabolism in the CNS [9,11]. Zinc cation is a small hydrophilic ion and can not freely cross-biological membranes by passive diffusion. Recently identified mammalian zinc transporters (ZnTs) are believed to participate in zinc trafficking across membranes in living cells [10,40]. Since 1995, seven members of the ZnT family have been cloned and characterized and are referred as to ZnT1–7. ZnTs are membrane-bound proteins with similar structures, consisting of six transmembrane domains and a histidine-rich loop, that is, supposed to be an important part of zinc binding. ZnT1 serves as a zinc ion efflux transporter of the cells and is expressed ubiquitously on the plasma membrane in a variety of tissues, including the brain [46,48,52]. ZnT2–4 are located on the vesicular membranes and have a similar structure and function, i.e. to transport zinc ions into intracellular vesicles. ZnT2 is abundantly expressed in intestine, kidney and testis and accumulate zinc ions in endosomal or lysosomal compartments [47]. ZnT3 is restrictedly expressed in the nervous system [34,58,61], and one of its important functions is presumably to sequester zinc ions into pre-synaptic vesicles of ZEN neurons, since no histologically detectable zinc can be found in ZnT3 knockout mouse brain [7]. The expression of ZnT4 is enriched in mammary gland, intestine, as well as in the brain [32,42,45], implying some specific roles in intracellular zinc homeostasis in these tissues. ZnT5 is homologous in the carboxyl-terminal portion with other members in the ZnT family, i.e. six membrane-spanning domains, but not in the amino-terminal portion, because it has a long amino-terminal portion, containing 410 amino acids. ZnT5 is responsible for transporting zinc into secretory granules in insulin-containing pancreatic β cells [35]. ZnT6 and ZnT7 are structurally and functionally similar but have differential tissue localizations. ZnT6 and ZnT7 facilitate the translocation of the cytoplasmic zinc into the Golgi apparatus [33,36]. In some pathological conditions, zinc ions and ZnTs may be involved in cell death and the plaque formation in Alzheimer's disease (AD) [26,38,39].

Several lines of studies showed that the cerebellum contains a significant amount of zinc ions [20] and the concentrations of zinc change dramatically during development and in pathological conditions in the cerebellum [30,51], however, very little is known about the zinc homeostasis in the cerebellum. Recently, it has been reported that a ZEN inhibitory GABAergic system exists in the mouse cerebellum [58]. In order to gain further insight on whether ZnT1, ZnT3, ZnT4 and ZnT6 are involved in zinc homeostasis in the cerebellar region, we explored cellular localization of ZnT1, ZnT3, ZnT4 and ZnT6 in the mouse cerebellar cortex.

2. Experimental procedures

Eight male BALB/c mice (22–25 g, 6-week-old, B and K Universal, Sollentuna, Sweden) were used. They were housed four in each cage on a 12 h light/dark cycle with food and water ad libtum. All procedures for animal handling were carried out in accordance with the permissions of the Animal Ethical Committees in Göteborg and Aarhus.

2.1. Tissue preparation and antibodies

Under pentobarbital anesthesia (50 mg/kg), three mice were perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brains were dissected and post-fixed in the same fixative overnight at 4 °C. For both the immunoperoxidase labeling and immunofluorescence staining, the brains were rinsed in PB, immersed in 0.01 M phosphate-buffered saline (PBS, pH 7.4), containing 20% sucrose and 0.1% sodium azide for 48 h at 4 °C and frozen with compressed CO_2. Transverse sections, 30 μm thick, were prepared in a cryostat. Sections were collected in PBS and kept in refrigerator at 4 °C. Single or double immunolabeling was carried out on free floating sections.

All ZnT antibodies used in this study were affinity-purified rabbit antisera specific for each ZnT protein.

(1) ZnT1 antiserum (dilution 1:500) was provided by Dr. W.F. Silverman, Department of Physiology, Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Israel [52].
(2) ZnT3 antiserum (dilution 1:100) was provided by Dr. R.D. Palmiter, Department of Biochemistry, University of Washington, USA [59,61].
(3) ZnT4 antiserum (dilution 1:20) [33,36].
(4) ZnT6 antiserum (dilution 1:25) [33,36].

(5) S-100β monoclonal antibody (dilution 1:4000) was purchased from Sigma–Aldrich, Stockholm, Sweden).
(6) Microtubule-associated protein 2 (MAP2) monoclonal antibody (dilution 1:100) was purchased from Sigma–Aldrich, Stockholm, Sweden).

Secondary antibodies and normal sera were purchased from Jackson ImmunoResearch, Pennsylvania, USA.

(1) Biotinylated goat anti-rabbit IgG (dilution 1:200).
(2) Fluorescein (DTAF)-conjugated streptavidin (dilution 1:800).
(3) Texas Red-conjugated donkey anti-mouse IgG (dilution 1:50).
(4) Normal goat serum (NGS).
(5) Normal donkey serum (NDS).

2.2. Immunoperoxidase procedures

Sections of mouse cerebellum were rinsed in 0.1 M Tris–HCl buffered saline (TBS, pH7.4). Endogenous peroxidase activity was quenched with 3% H_2O_2 in 20% methanol for 15 min. Following several rinses with TBS, containing 0.25% Triton X-100, sections were incubated overnight at 4 °C with primary antibodies specific against ZnT1, ZnT3, ZnT4 and ZnT6, respectively. The antibodies were diluted in TBS, containing 3% NGS, 1% bovine serum albumin (BSA) and 0.25% Triton X-100. After several rinses, sections were incubated in biotinylated goat anti-rabbit IgG at 1:200 for 1 h at room temperature. They were then rinsed and incubated with streptavidin-HRP (1:100) for 2 h at room temperature. Sections were rinsed in TBS and incubated in 0.025% 3,3-diaminobenzidine tetrahydrochloride plus 0.0033% H_2O_2 in TBS for 10–15 min. All procedures were carried out with gentle shaking. The stained sections in free floating incubations were washed in TBS, mounted on glass slides, dehydrated with ethanol, cleared with xylene and cover-slipped with DEPEX. Sections were examined in a light microscope (Nikon Optiphot) equipped with the Easy Image Analysis System (Bergström Instruments AB, Göteborg, Sweden). Grayscale images were collected and further processed with Adobe Photoshop.

2.3. Immunofluorescence procedures

All immunofluorescence procedures were performed at room temperature. The normal sera, primary and secondary antibodies were all diluted in 0.01 M PBS, containing 1% BSA and 0.25% Triton X-100.

For single labeling with the ZnT antibodies, cryostat sections were pre-incubated in NGS at 1:50 for 1 h. Incubations with rabbit anti-ZnT1, ZnT3, ZnT4 or ZnT6 were carried out overnight. Following three rinses in 0.01 M PBS, biotinylated goat anti-rabbit IgG was applied for 2 h to label primary antibodies. Sections were then rinsed and incubated in DTAF-conjugated streptavidin for 2 h. After rinsing with PBS and distilled water, sections were mounted in an anti-fading mounting medium.

Double labeling with S-100β or MAP2 monoclonal antibody and one of the rabbit anti-ZnT antibodies was carried out on consecutive sections of the cerebellum. Sections were pre-incubated with NGS and NDS for 1 h, incubated in a mixture of primary antibodies against mouse anti-S-100β or MAP2 together rabbit anti-ZnT1, ZnT3, ZnT4 or ZnT6 overnight. After rinsing, sections were incubated for 2 h with biotinylated goat anti-rabbit IgG to label one of the primary antibodies of ZnTs. Sections were rinsed in PBS and incubated for 2 h with a mixture of DTAF-conjugated streptavidin (for labeling ZnT1, ZnT3, ZnT4 and ZnT6) and Texas Red-conjugated donkey anti-mouse IgG (for labeling S-100β or MAP2). After rinsing, sections were mounted with anti-fading medium. Control sections, included in every incubation series, were incubated with normal sera instead of the primary antibodies, followed by all subsequent incubations as described above. No specific staining was observed.

After immunofluorescence labeling, sections of mouse cerebellum were examined with a confocal laser scanning microscope (CLSM) with a krypton/argon laser (MRC 1024, BIO-RAD). The excitation filters for DTAF (488 nm) and Texas-Red (568 nm) were used. Images were collected using Kalman mode scanning (10 scans) from single or dual channel scanning. Colocalization was verified by a sequential scan between the two channels. Images were processes using an Adobe Photoshop program.

3. Results

3.1. Single labeling: the distribution of ZnT1, ZnT3, ZnT4 and ZnT6 in the cerebellar cortex

In general, ZnT1, ZnT3, ZnT4 and ZnT6 were present in the neuronal cell bodies, nerve terminals, glial cell bodies and their processes with varying density and intensity in different layers of the cerebellar cortex. Since, both immunoperoxidase and immunofluoresence showed the same distribution patterns of ZnT1, ZnT3, ZnT4 and ZnT6 in the cerebellar cortex, here we describe the immunoreactivity of each ZnT based on confocal microscopic observations. The overall distribution of ZnTs immunofluorescence was summarized in Table 1. The intensity of the immunofluorescence was rated from negative, weak, moderate to strong.

ZnT1: ZnT1-containing structures were localized in the whole cerebellar cortex. In the molecular layer, ZnT1 immunofluorescence was observed throughout the superficial stratum layer to the deeper layer (Fig. 1a–a′), while it was completely absent from the cell bodies. Following the ZnT1-positive processes from the molecular layer to the Purkinje cell layer, the intensity of ZnT1 immunoreactivity increased in the small cell bodies, surrounding the Purkinje cells. Double labeling between ZnTs and S-100β suggested that these ZnT1-positive cells were Bergman glial cells

Table 1
Immunofluorescence of ZnT1, ZnT3, ZnT4 and ZnT6 in the cerebellar cortex*

ZnT	Molecular layer		Purkinje cell layer		Granular layer	
	CB	GF	PC	BG	CB	NT
ZnT1	–	++	++	+++	++	–
ZnT3	–	++	+	++	–	+++
ZnT4	–	++	–	+++	–	+
ZnT6	–	++	–	++	–	–

Abbreviations: BG, Bergman glia; CB, cell body; GF, glia fiber; NT, nerve terminal; PC, Purkinje cell; ZnT, zinc transporter.

* The intensity of immunoreactivity was graded as follows: (+++), strong fluorescence; (++), moderate fluorescence; (+), weak fluorescence; (–), negative.

(Fig. 2a–a″). Purkinje cells also showed weak to moderate immunofluorescence of ZnT1. No nerve terminals with ZnT1 immunofluorescence could be seen in the Purkinje cell layer or in the granular layer. A few neuronal somata probably a subset of granule cells, were ZnT1-positive (data not shown).

ZnT3: In the molecular layer and the Purkinje cell layer, ZnT3 immunoreactivity was found in the processes and the cell bodies of the Bergman glial cells. However, some dot-like structures that may be nerve terminal varicosities were also ZnT3-positive in these two layers (Fig. 1b–b′; Fig. 2b–b″). Based on our previous studies, these ZnT3-positive terminals probably belong to the satellite and basket cells in the superficial stratum layer and the deeper layer of the molecular layer, respectively [58]. ZnT3 was very weakly present or absent in the Purkinje cell bodies. In the granular cell layer, ZnT3 was distributed with very high intensity in the terminals on the glomeruli (Fig. 1b–b′, Fig. 2b–b″). These ZnT3-positive structures probably represent nerve terminals of Golgi cells, because they were almost all positive for glutamate decarboxylase (GAD), a marker for GABAergic neurons [58]. Neither neuronal nor glial cell bodies were ZnT3-positive in the granular cell layer.

ZnT4: ZnT4 was abundant in the molecular layer and the Purkinje cell layer, but was weakly distributed in the granular layers. The Bergman glial cell bodies and their processes showed strong ZnT4 immunoreactivity (Fig. 1c–c′). However, neither the neuronal somata, including the Purkinje cells, nor the nerve terminals were ZnT4-positive in the Purkinje cell layer or the molecular layer. In the granular cell layer, some dot-like structures with weak intensity of ZnT4 immunofluorescence could be seen, and these ZnT4-positive elements were presumed to be the Golgi cell terminals, based on their size and position [58]. No other ZnT4-positive elements could be seen in the granular layer.

ZnT6: The distribution of ZnT6 was similar to that of ZnT4 in the molecular layer and the Purkinje cell layer. ZnT6 immunoreactivity was observed in the Bergman glial cell bodies and their processes with strong intensity (Fig. 1d–d′, Fig. 2d–d″). ZnT6 was absent in the Purkinje cell bodies and the neuronal terminals. No ZnT6-positive structures could be seen in the granular cell layer.

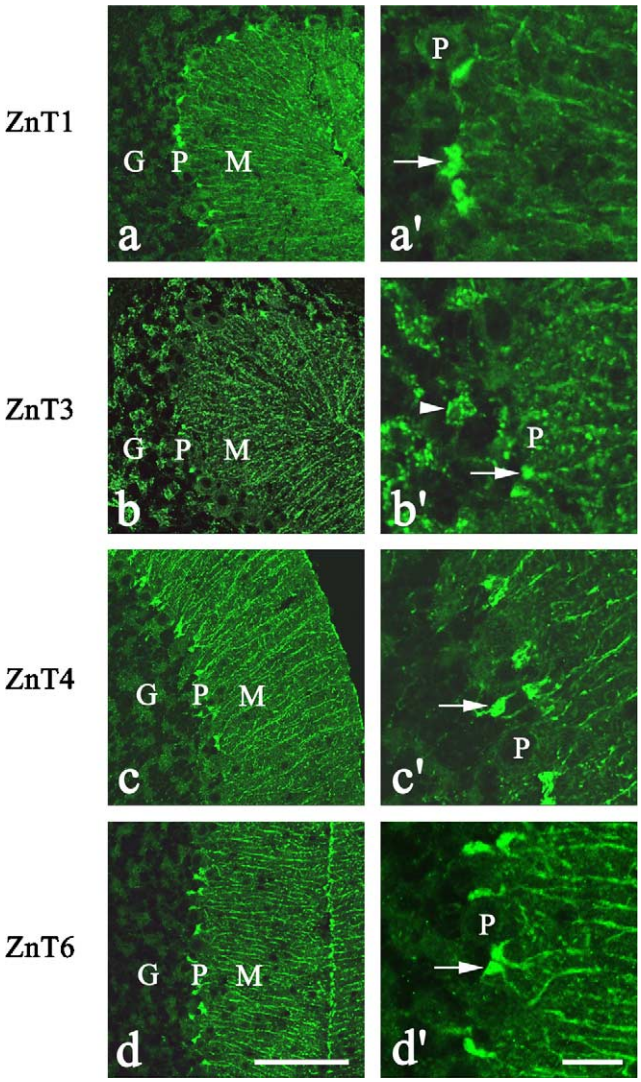

Fig. 1. Single immunofluorescence staining of ZnT1 (a–a′), ZnT3 (b–b′), ZnT4 (c–c′) and ZnT6 (d–d′) in the mouse cerebellar cortex. Four ZnTs were all abundantly expressed in the Bergman glia (arrows) in the Purkinje cell layer (P) and their fibers in the molecular layer (M). However, the distribution of the tested ZnTs was different in neuronal structures. ZnT1 was expressed in the Purkinje cell bodies with a moderate immunofluorescence (a–a′). ZnT3 appeared in the neuronal terminals in the whole cerebellar cortex, especially in the granular cell layer (G) (arrow head in b′), whereas ZnT4 showed a weak immunostaining in the neuronal terminals in the granular layer (c–c′). ZnT6 was not present in any neuronal structures in the cerebellar cortex (d–d′). Scale bars = 80 μm (a–d); 20 μm (a′–d′).

3.2. *Double labeling: ZnTs overlapped with S-100β in the Bergman glia*

Double labeling of S-100β or MAP2 and ZnT1, ZnT3, ZnT4 or ZnT6 was performed to analyze the glial and/or neuronal cellular distribution of ZnT1, ZnT3, ZnT4 and ZnT6 in the cerebellar cortex. Generally, the patterns of

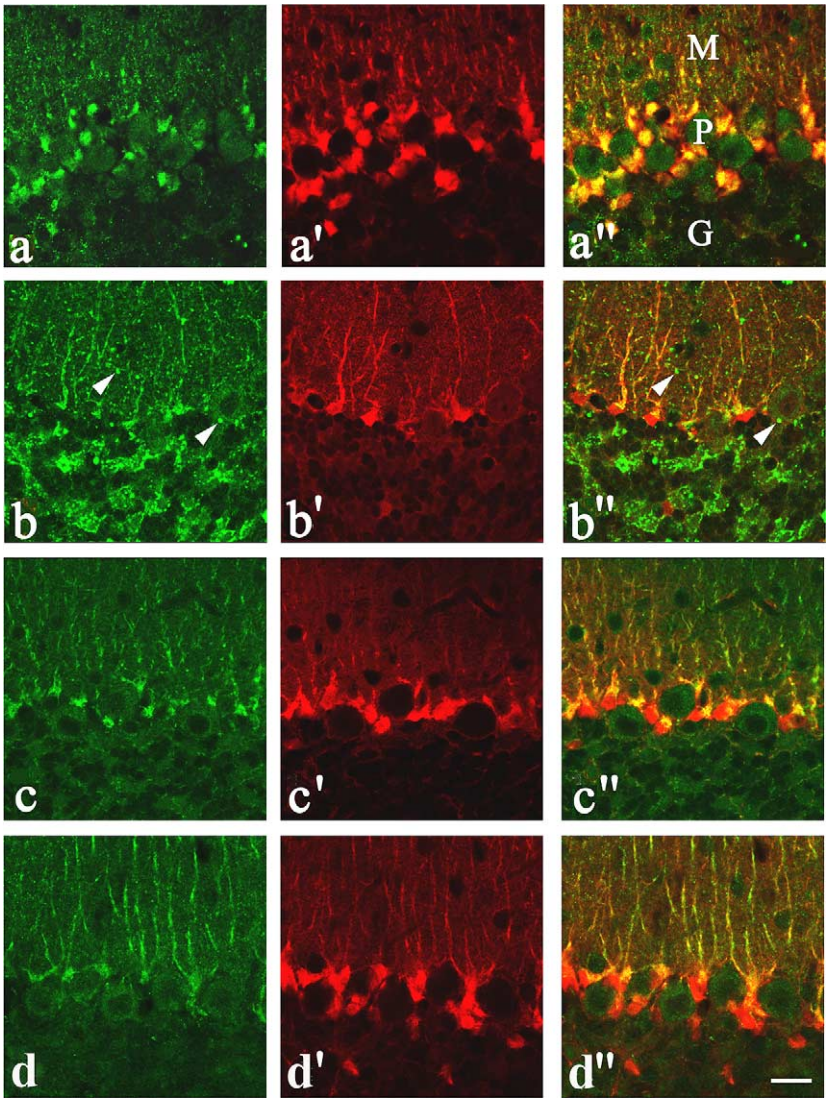

Fig. 2. Double immunofluorescence labeling between ZnT1 (a), ZnT3 (b), ZnT4 (c) or ZnT6 (d) and S-100β (a′, b′, c′, d′) in the mouse cerebellar cortex. (a–a′) Cofocal images showed that the S-100β-positive Bergman glia cells contain ZnT1 immunoreactivity. Moreover, the Purkinje cell bodies were present moderate level of ZnT1, but devoid of S-100β immunofluorescence. M, molecular layer; P, Purkinje cell layer; G, granular layer. (b–b″) The Bergman glia showed both ZnT3 (b) and S-100β (b′) with extensive immunoreactivity. Neuronal terminals with ZnT3 but not S-100β immunofluorescence could be observed in the granular layer, and in the molecular and Purkinje cell layer as well (arrow heads). (c–c″, d–d″) The S-100β-positive Bergman glia cells were also ZnT4-(c) and ZnT6-(d) positive. In the granular layer, nerve terminals showed weak immunofluorescence of ZnT4, but not ZnT6. Scale bar = 20 μm.

ZnTs and S-100β or MAP2 immunofluorescence were different in the cerebellar cortex. However, the small cells surrounding the Purkinje cells were both ZnTs and S-100β positive, with a strictly superimposed pattern (Fig. 2). Based on the size, location and immunostaining features, these ZnTs-containing cells were judged to be the Bergman glial cells. Furthermore, double labeling of MAP2 and ZnTs showed that no colocalization could be found in the molecular or the Purkinje cell layers, suggesting that the processes with strong ZnTs-immunoreactivity in these two layers do not belong to neuronal elements but to the Bergman glia (Fig. 3a–a″–c–c″). It should be pointed out that the Bergman glial cells in the cerebellar cortex contained all ZnTs tested in this experiment, i.e. ZnT1, ZnT3, ZnT4 and ZnT6, with strong immunofluorescence.

4. Discussion

In the present study, the distribution of four members of the ZnT family, ZnT1, ZnT3, ZnT4 and ZnT6, which are abundantly expressed in brain [32,33,52,61], was investigated in the cerebellar cortex. In general, our results demonstrated that all ZnTs were expressed in the Bergman glial cells, but were different in neuronal structures, suggesting (1) that the Bergman glia cells are important for zinc metabolism in the mammalian cerebellum and (2) that the differential

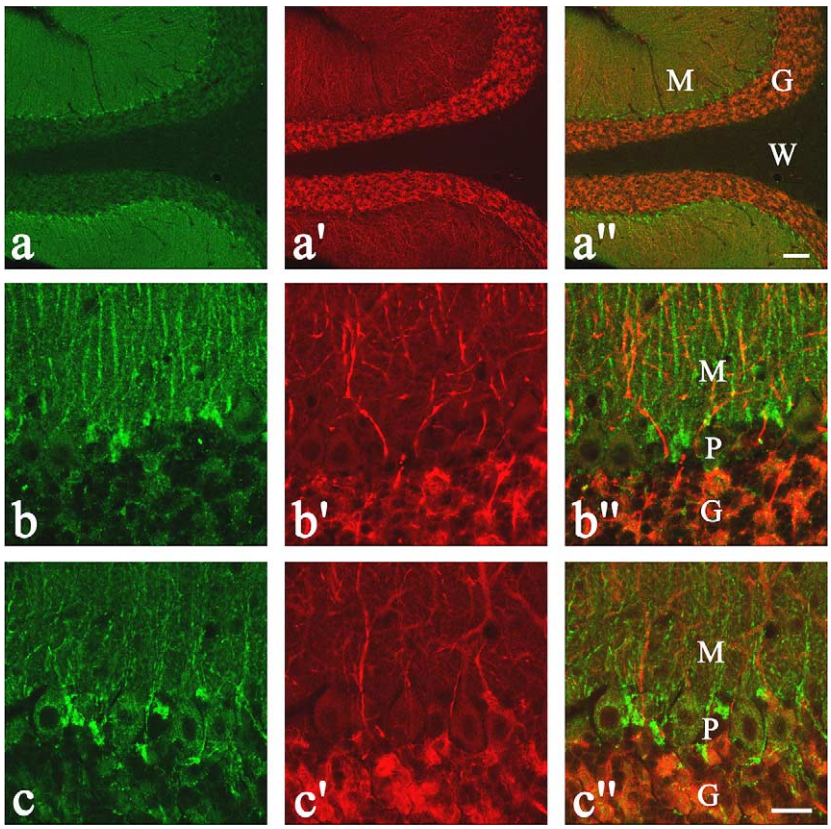

Fig. 3. Double immunofluorescence labeling of ZnT1 (a, b) or ZnT4 (c) with MAP2 (a′–c′) in the mouse cerebellar cortex. (a–a′′) Different immunostaining pattern of ZnT1 (a) and MAP2 (a′) could be seen in the cerebellar cortex. (b–b′′, c–c′′) High magnification images showed there was no colocalization between ZnT1 (b) or ZnT4 (c) and MAP2 (b′, c′) in the molecular layer and the Purkinje cell layer, suggesting that the ZnT1- and ZnT4-positive fibers in molecular and the Purkinje layers were not dendrites. M, molecular layer; P, Purkinje cell layer; G, granular cell layer; W, white matter. Scale bars = 50 μm (a–a′′); 20 μm (b–b′′, c–c′′).

distribution of ZnTs in the neuronal elements probably imply functional differences in zinc associated neuronal events between these proteins.

The most interesting finding in this study is that the Bergman glia cells contain all the four ZnTs tested. Both immunoperoxidase and single immunofluorescence revealed that numerous small cells surrounding the Purkinje cells, and their radial fibers in the molecular layer, were immunopositive to ZnT1, ZnT3, ZnT4 and ZnT6. In order to precisely verify the immunolabeled cells and the radial fibers, double immunofluorescence was performed between one of the ZnTs and one of the established markers of astrocytes, S-100β [27,50], and of neuronal dendrites, MAP2 [41,55]. As expected, ZnTs and S-100β was colocalized largely in the Bergman glial cells and their fibers. No colocalization between ZnTs and MAP2 could be seen in the dendrites in the molecular layer, indicating that the S-100β-positive Bergman glial cells contain the ZnT proteins. To our knowledge, this for the first time demonstrates that the glial cells contain immunoreactivities of so many zinc transporter proteins.

It is worth noting that co-existence of different ZnT proteins was only found in the Bergman cells, but not the other cerebellar structures, in agreement with previous observations that several ZnTs were co-expressed in the same tissue [49]. This observation suggests a complicated zinc homeostasis in the Bergman glia. It is known that ZnTs have high degree of sequence and structure homology, but they display distinct intracellular localizations and play different roles in maintaining zinc concentrations in cell [1,10]. ZnT1 is a zinc exporter, transporting zinc from the cytoplasm to extracellular spaces [48], while ZnT3, ZnT4 and ZnT6 are responsible for transporting zinc into vesicles in cell [7,32,33,61]. Therefore, we hypothesis that the Bergman glia may be an important zinc pool in the cerebellar cortex and that this zinc pool may participate in physiologically relevant changes in free zinc ions or changes in zinc bound to specific ligands.

Our results show that, except the co-expression in the Bergman glia, ZnT1, ZnT3, ZnT4 and ZnT6 were distributed in different neuronal structures, in agreement with previous reports, which have shown that ZnT1 immunoreactivity was observed in the Purkinje cells in the cerebellum [46,52] and that ZnT3 immunoreactivity was located in the nerve terminals throughout the cerebellar cortex probably GABAergic terminals as they contain GAD [58]. However, the intense expression of ZnT3 immunoreactivity in the astrocyte, the Bergman glial cell, suggests that ZnT3 may also be involved in zinc transportation into vesicular compartments in non-neuronal cells. Furthermore, our results show that ZnT-4

immunofluorescence was weakly expressed in the neuronal terminals in the granular layer in the cerebellum, suggesting this protein may be involved in transport of zinc ions into synaptic vesicles.

Previous studies showed that zinc deficiency induced alterations in the postnatal development of the cerebellar cortex [17–19]. Recent studies have demonstrated that Purkinje cells contain MT-III, a brain-specific member of the metal-binding proteins MT family, suggesting that MT-III may play a role in protecting Purkinje cells from ischemic insult by reducing neurotoxic zinc levels [63]. The other two members of the MT family, MT-I/II, are abundant in astrocytes, and they are involved in zinc metabolism in the CNS. In situ hybridization study revealed that MT-I mRNA was located in several brain areas with the highest level in the cerebellum [28]. Analysis of Alzheimer's disease (AD) brain sections revealed high expression of MT-I/II in the granular layer of the cerebellum [65]. Taken together with the present results of the overlapping presence of many ZnTs in the Bergman glia, but differential distribution of ZnTs in neuronal structures in the cerebellar cortex, it is likely that ZnTs, MTs and other zinc associated proteins form a complicated network, which participates in zinc associated functions and homeostasis between neurons and glial cells.

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